

***Paraochoterenella javanensis* gen. et sp. n. (Filarioidea: Onchocercidae) from *Rana cancrivora* (Amphibia: Anura) in West Java, Indonesia**

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ABSTRACT: *Paraochoterenella javanensis* gen. et sp. n. (Filarioidea: Onchocercidae) is described from the mesentery of the frog *Rana cancrivora* Gravenhorst in West Java, Indonesia. *Paraochoterenella javanensis* presently represents the only species in this newly created genus. Three of 13 frogs contained mature male and female worms and microfilariae. *Paraochoterenella* is distinguished from the other 4 genera, *Foleyellides* Caballero, *Ochoterenella* Caballero, *Madochotera* Bain and Brunhes, and *Paramadochotera* Esslinger in the subfamily Waltonellinae Bain and Prod'hon by the presence of cuticularized parastomal structures in both sexes, a distinct cuticularized buccal capsule, the lack of both lateral and caudal alae, and the presence of scattered (nonoriented) minute bosses on the cuticle of the midbody region. The microfilariae are unsheathed and slightly narrowed at the caudal extremity, with a 10:1 length to width ratio. *Paraochoterenella* represents the second genus in the subfamily Waltonellinae present in southern Asia and the first report of a filarial species in the subfamily from an Indonesian amphibian. A revised key to the genera is presented in light of this new addition to the subfamily.

KEY WORDS: Filarioidea, Onchocercidae, Waltonellinae, *Paraochoterenella javanensis* gen. et sp. n., taxonomic key, *Rana cancrivora*, Amphibia, Anura, Ranidae, morphology, Java, Indonesia.

The majority of the species contained in the subfamily Waltonellinae Bain and Prod'hon, 1974 (Filarioidea: Onchocercidae) have been described from the Western Hemisphere, particularly neotropical areas. Esslinger (1986a, b) provided redescrptions of type material of *Ochoterenella* Caballero, 1944, and *Foleyellides* Caballero, 1935, and revisions of the 4 related genera in the subfamily. Species previously assigned to the genus *Waltonella* Schacher, 1975, were transferred into the genera *Foleyellides* and *Ochoterenella*, the name *Waltonella* was placed as a junior synonym of *Foleyellides*, and the subfamily designation Waltonellinae was retained (Esslinger, 1986a, b). Members of this filarial assemblage have only been found in the body cavities of anuran amphibians, with the exception of 1 subcutaneous parasite, *Foleyellides confusa* (Schmidt and Kuntz, 1969; see Anderson and Bain, 1976). The subfamily members are parasites of toads and frogs in the families Bufonidae, Leptodactylidae, Racophoridae, and Ranidae.

Three of 13 frogs identified as *Rana cancrivora* Gravenhorst, 1829 (Anura: Ranidae), and collected from Bekasi, West Java, Indonesia, were examined and discovered to harbor adults and microfilariae of an *Ochoterenella*-like nem-

atode. Microfilariae found in the blood and adult male and female worms removed from the mesentery belong to a previously unknown genus and species as described herein.

Materials and Methods

Live frogs were obtained from a local food dealer residing in Jakarta. All had been captured from the same locality along drainage ditches, approximately 5 km east of the city of Jakarta proper. Live adult worms were removed from the mesentery, relaxed in 0.6% saline solution, fixed in hot 70% ethanol, and preserved in 70% ethanol/5% glycerine. All specimens were cleared and temporarily mounted and examined in lactophenol. Microfilariae were obtained from blood. Thick blood films were processed with Giemsa's stain diluted 1:15 with pH 7.2 sodium phosphate buffer for 15 min. Drawings and measurements were made with the aid of a camera lucida. All measurements are expressed as means followed by the range in parentheses and are given as length by width in micrometers (μ m) unless otherwise indicated.

Results

***Paraochoterenella* gen. n.**

DIAGNOSIS: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Waltonellinae Bain and Prod'hon, 1974. Cephalic end with pair of lateral flap-like cuticularized parastomal structures. Cephalic plate with lateral axis slightly longer than dorsoventral axis; 4 pairs cephalic papillae, broad basally and tapered with nonarticulated distal portion. Distinct cuticularized

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buccal cavity. Lateral and caudal alae lacking. Esophagus divided into a short anterior muscular region and a long, wider posterior glandular portion (muscular to glandular ratio: 6.1). Vulva in posterior region of glandular portion of esophagus near esophageal-intestinal junction. Two pairs of preanal, 4 pairs of postanal papillae. Cuticular bosses minute ($<2-3\ \mu\text{m}$), nonbacillary in appearance, with irregular scattered distribution from anterior cephalic to caudal region just beyond anus. Microfilaria unsheathed, slightly tapered at caudal end. Parasite of body cavity of frog (Ranidae) in tropical Southeast Asia.

TYPE AND ONLY SPECIES: *Paraochoterella javanensis* sp. n. from *Rana cancrivora* (West Java, Indonesia).

***Paraochoterella javanensis* sp. n.**
(Figs. 1–17)

Description

GENERAL: Adult worms small and filiform, yellow to white. Anterior end blunt. Posterior end conical and blunt. Female 2.7 times as long as male. Four pairs of submedian papillae and 2 lateral cephalic amphids (Figs. 1, 9). A pair of lateral flap-like cuticularized parastomal structures (Fig. 9). Cuticularized buccal cavity (Figs. 8, 10). Esophagus divided into anterior muscular and posterior glandular portion, the latter being longer and wider, with ratio 6.1 (Fig. 3). Vulva near esophagointestinal junction. Nerve ring at posterior half of muscular region of esophagus. Lateral and caudal alae absent. Two pairs of preanal, 4 pairs of postanal papillae; first 2 pairs well anterior to anus (Fig. 7). Larger (left) spicule long, with very slender shaft (Fig. 6). Cuticle transversely striated (Fig. 2), with minute cuticular bosses present in nonoriented, irregular distribution (Figs. 11–17).

MALE (based on 3 mature specimens: 1 complete and 2 partial): Body 8.2 mm long by 52 wide at level of head; width increasing posteriorly, 104 at level of nerve ring, 156 at level of esophageal-intestinal junction, and 176 (160–200) at midlevel of body, gradually decreasing posteriorly, 71 (65–80) at level of anus and 48 (45–52) at midlevel of tail. Esophagus 1.7 mm, distinctly divided into anterior muscular portion 187 by 26 and posterior glandular region 1.51 mm by 78. Nerve ring 130 from cephalic end. Tail curved ventrally, 80 (78–85). Spicules thin, unequal in length, and dissimilar in appearance.

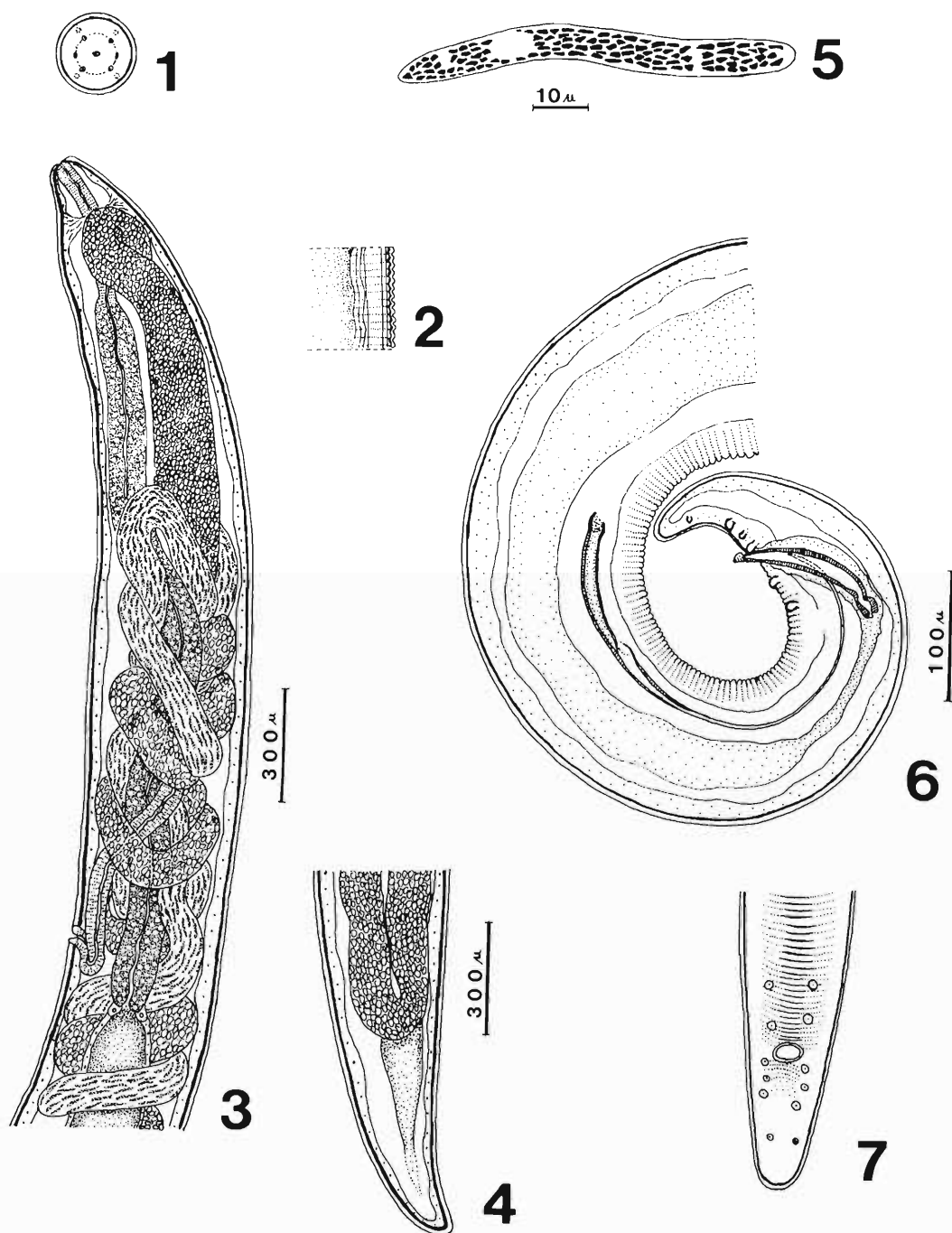
Larger (left) spicule 389 (380–398), composed of 3 sections: a thick-walled cylindrical section 105 (99–117) having an open capitulum at proximal position, a thin-walled midsection 27 (25–39), and a long narrow rod-like distal portion 250 (234–260), ending in pointed tip (Fig. 6). Smaller (right) spicule 106 (104–110), ending in hook-like tip. Left to right spicule ratio 3.7 (3.4–3.8):1. Gubernaculum absent. Cuticle at cervical, midbody, area rugosa, and anal region (Figs. 14–17) with scattered (nonoriented) minute bosses ($<2-3\ \mu\text{m}$).

FEMALE (based on 3 gravid specimens): Body 22.0 (21.8–22.5) mm by 63 (60–70) at level of head, width increasing posteriorly, 120 (110–130) at level of cervical region, 197 (190–200) at level of nerve ring, 410 (380–430) at level of vulval opening, 413 (400–440) at level of esophageal-intestinal junction, 477 (450–520) at midbody, gradually decreasing to 159 (156–166) at anal opening and 80 (70–90) at midlevel of tail. Esophagus 2.31 (2.15–2.50) mm, distinctly divided into anterior muscular region 325 (280–370) by 41 (31–47) and posterior glandular region 1.97 (1.87–2.13) mm by 120 (104–130), muscular to glandular ratio 6.1 (5.7–6.7). Nerve ring 152 (150–156) from cephalic end. Vulva, opening as transverse slit, anterior to esophageal-intestinal junction, 2.15 (2–2.26) mm from cephalic end (Fig. 3). Vagina directed anteriorly and flexing and looping posteriad before receiving bifurcated uterus at varying distance below vulva. Uteri paired, loosely entwined, joining oviduct and extending anteriorly to within 277 (140–340) from cephalic end; posterior coil extending to within 277 (140–300) from caudal end. Viviparous. Tail 230 (150–286) with blunt end (Fig. 4). Cuticular bosses as in male, minute and nonoriented (Figs. 11–13).

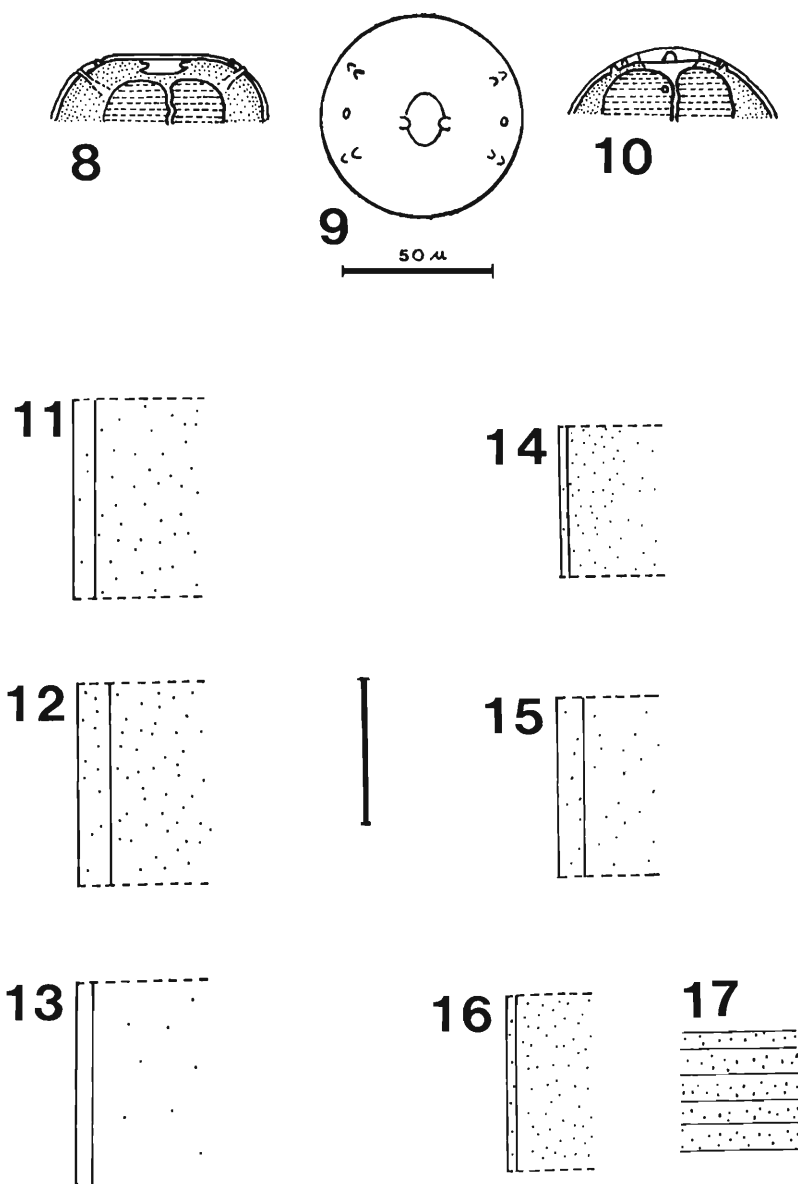
MICROFILARIA (based on 20 specimens): Body stout and elongated, unsheathed 80.8 (70–92) long. Caudal extremity slightly narrowed, not bulbous or rounded (Fig. 5). Width at level of first cephalic nucleus 6.7 (5–7), nerve ring 8.6 (8–9), excretory pore 8.5 (8–9), anal pore 7.0 (6–8), and at level of last caudal nucleus 2.3 (2–3). One single tail nucleus. Nerve ring 21.6 (19–25), excretory pore 52.3 (43–57), and anal pore 68.7 (56–78) from cephalic end. Cephalic space length to width ratio, 0.4 (0.3–0.6):1.

Taxonomic summary

TYPE HOST: The mangrove frog, *Rana cancrivora* Gravenhorst, 1829.



Figures 1-7. Adults and microfilaria of *Paraochoterenella javanensis* gen. et sp. n. 1. Generalized en face view of female showing arrangement of 4 pairs of submedian papillae and 2 lateral amphids. 2. Transversely striated cuticle of female. 3. Anterior region of female, lateral view. 4. Caudal end of female, lateral view. 5. Microfilaria from blood. 6. Caudal end of male, lateral view showing left and right spicules, cloaca, and caudal papillae. 7. Caudal end of male, ventral view showing arrangement of caudal papillae. All scale bars in μm .



Figures 8–17. *Paraochoterenella javanensis* gen. et sp. n. 8. Cephalic extremity of female, dorsal view showing cuticularized buccal capsule. 9. Cephalic extremity of female, en face view showing arrangement of papillae, amphids, and parastomal structures. 10. Cephalic extremity of female, lateral view. 11, 12, 13. Minute bosses on female, lateral views of cervical region (11), midbody (12), and anal region (13). 14, 15, 16. Minute bosses on male, lateral view, cervical region (14), midbody (15), and anal region (16). 17. Detail of area rugosa of male, ventral view. Scale bars = 50 μ m (Figs. 8–10) and 200 μ m (Figs. 11–17).

TYPE LOCALITY: Indonesia, West Java, Bekasi.

SITE OF INFECTION: Mesentery.

DATE OF COLLECTION: AUGUST 1990.

DEPOSITED SPECIMENS: Holotype male,

USNPC 82165; allotype female, USNPC 82166; paratypes, 2 females, USNPC 82167 in 70% ethanol/5% glycerine; 1 blood slide, Giemsa-stained microfilariae (syntypes), USNPC 82168, deposited in the U.S. National Parasite Collec-

tion (USNPC), Beltsville, Maryland. Preserved frog specimens have been retained at U.S. Naval Medical Research Unit-2 in 10% formalin.

ETYMOLOGY: The specific epithet is derived from the type locality of the new species.

Remarks and Discussion

The finding of *Paraochoterenella javanensis* sp. n. from the mangrove frog, *Rana cancrivora*, in Bekasi, West Java, represents the first report of a filarial species in the subfamily Waltonellinae from an Indonesian amphibian. The subfamily members are parasites of toads and frogs in Bufonidae, Leptodactylidae, Racophoridae, and Ranidae. Of the Waltonellinae, only *Foleyellides* and *Paraochoterenella* have been definitively described from ranid frogs (Anderson and Bain, 1976). Geographically, *Ochoterenella* appears restricted to the Neotropical Region. Species of *Foleyellides* have been recorded predominantly in the Western Hemisphere, whereas species of both *Madochotera* Bain and Brunhes, 1968, and *Paramadochotera* Esslinger, 1986, have been described only from Madagascar (Bain and Brunhes, 1968, Esslinger, 1986a).

Based on described morphological measures and structures of adults and microfilariae, together with information on definitive hosts and geographic localities, the generic name *Paraochoterenella* is proposed to accommodate the new species, *P. javanensis*. The specific characters deemed important in distinguishing genera in the subfamily Waltonellinae, as revised by Esslinger (1986a, b), are the presence or absence of caudal alae and parastomal structures, the appearance and arrangement of the cuticular bosses, and the morphology of the microfilariae. *Paraochoterenella* gen. n. shares various characters with the other 4 genera, including paired cephalic papillae without articulated tips, cuticularized parastomal structures, the absence of lateral and caudal alae, a distinct buccal formation, the vulva near the base of the glandular esophagus, a thin, elongated (left) spicule shaft, and being a parasite of an anuran (Anderson and Bain, 1976). The principal characters that separate *Paraochoterenella* from other genera are the unsheathed microfilaria and the appearance and arrangement of the cuticular bosses.

Of the 4 previously recognized genera in the subfamily, *Paraochoterenella* appears most closely aligned with *Ochoterenella*. However, Esslinger (1986a, 1988) concluded that all *Och-*

oterenella species are a morphologically uniform group, restricted to the Neotropical Region. With the exception of only 2 species, both recovered from leptodactylid frogs, all have been found only in the toad *Bufo marinus* Linnaeus, 1758 (Esslinger, 1988). Before Esslinger's (1986a) detailed reassessment, only 3 species of *Ochoterenella* Caballero, 1944 (Caballero, 1944; Johnston, 1967), in the subfamily (Bain and Prod'hon, 1974) had been assigned to the genus. *Ochoterenella digiticauda* Caballero, 1944, has been found in Mexico, Guatemala, and Paraguay (Lent et al., 1946; Yamaguti, 1961). *Ochoterenella papuensis* Johnston, 1967, found in the frog *Platymantis* (= *Cornufer*) *papuensis* Meyer, 1875 has been reported only from New Guinea (Johnston, 1967), whereas *Ochoterenella guibei* Bain and Prod'hon, 1974 from a racophorid frog, appears in Madagascar.

Esslinger (1988) included 14 members in *Ochoterenella*, partially the result of a previous transfer of 8 species in the genus *Waltonella* to *Ochoterenella* (Esslinger, 1986a). *Ochoterenella guibei* from Madagascar was placed in a new genus *Paramadochotera*. *Ochoterenella papuensis* from New Guinea, as described by Johnston (1967), was considered incertae sedis and was removed from the genus until more material could be fully described (Esslinger, 1986a). An incompletely described *Ochoterenella* species from northern Viet Nam (Moravec and Sey, 1985) is also considered incertae sedis for the present. Specific identification of the Viet Nam filariid was not possible because of the absence of males and the poor condition of the 5 female specimens. It is also noted that both Asian populations assigned to *Ochoterenella* lacked a distinct buccal cavity and the microfilariae were unsheathed, characters present in all known members of *Ochoterenella* (Esslinger, 1986a, b). However, because *O. papuensis* and the Viet Nam specimens represent the only purported members of the genus described from the Asian region, both are briefly mentioned in this discussion.

Paraochoterenella javanensis can be distinguished from *O. digiticauda* (the type species), *O. papuensis*, and *Ochoterenella* from Viet Nam (VN) by the following characters: adult male and female worms are shorter in body length (except VN sp.), the longer (left) spicule is nearly 4 times as long as the right (male not described for VN sp.), and the spicule ratio (3.7:

1) is greater (1.7:1 and 2:1, respectively). Unlike all recognized members of *Ochoterenella*, the microfilariae of *P. javanensis* are unsheathed in blood. On average, the microfilariae are shorter in length, but have a distinctly smaller length to width body ratio (10:1) compared to *O. digiticauda* and *O. papuensis* (~30:1) and *Ochoterenella* VN (~20:1). The tip of the microfilaria tail is slightly narrowed versus the rounded, usually bulbous appearance in *O. digiticauda*. The VN microfilariae were described as unsheathed.

In *Ochoterenella*, the appearance and length of individual bosses in both sexes are considered consistent within a species (Esslinger, 1986a). Depending on the site of measurement, the individual bosses of *O. digiticauda* ranged in mean size from 8.7 μ m at the midbody to 4 μ m at the midportion of the area rugosa (Esslinger, 1986a). The scattered appearance of minute bosses on the cuticle, in the size range of 2–3 μ m, clearly set *P. javanensis* apart from *Ochoterenella* species. *Ochoterenella papuensis* female worms apparently lack surface tubercles, and tubercles were not described for the VN specimen.

The arrangement of male preanal and caudal papillae differs among the 4 descriptions. *Paraochoterenella javanensis* has 2 pairs of preanal and 4 pairs of postanal papillae, and *O. digiticauda* has 2 pairs of preanal and 3 pairs of postanal (Caballero, 1944) or 1 pair of preanal and 3 pairs of postanal papillae as reported by Lent et al. (1946) and Esslinger (1986a). *Paraochoterenella javanensis* also lacks a median ventral preanal plaque. *Ochoterenella papuensis* has 2 single preanal papillae in tandem, 2 adanal papillae, and 3 pairs of postanal papilla. *Paraochoterenella javanensis* has 4 pairs of anterior submedian papillae, 2 lateral cephalic amphids, and parastomal structures similar to those of *O. digiticauda* and the VN *Ochoterenella* sp. The position of the vulva is very near the glandular esophagointestinal junction, similar to that in *O. digiticauda*. In *O. papuensis*, the vulva was indistinct, lying slightly behind the musculoglandular esophageal junction, whereas *Ochoterenella* (VN) had it positioned at about the mid-point of the glandular esophagus. Unlike all members of *Ochoterenella*, *P. javanensis* has a distinct cuticularized buccal capsule, presently found elsewhere only in the genus *Paramadochotera*.

Paraochoterenella represents a second genus

in the subfamily present in southern Asia. The distribution and host range of this monotypic genus is not known. To date, only *Foleyellides* (= *Waltonella*) *confusa* from the Philippines and *Foleyellides* (= *Waltonella*) *malayensis* (Petit and Yen, 1979) from peninsular Malaysia have been described. It is possible that specimens described from Viet Nam and New Guinea may be members of *Paraochoterenella*, because their microfilariae also lacked a cuticular sheath; however, a decision regarding this possibility awaits full descriptions. The limited number of species described outside the Western Hemisphere may be more reflective of the lower relative number of investigations on amphibians and their nematodes from other areas of the world (Esslinger, 1986b). Given the wide range and species diversity of anuran amphibian species present in the Asian Region, this would not seem unreasonable.

Nothing is known of the biology or transmission of *Paraochoterenella javanensis*. Larval stages have only been described from a few species of *Foleyellides* (= *Waltonella*), based primarily on experimental infections (Bain and Chabaud, 1986). Larval stage development has been observed in adipose and muscle tissue of mosquitoes. Likewise, the natural intermediate hosts of Waltonellinae are poorly known except for a few *Foleyellides*. Vectors are presumed to be blood-feeding dipterans, most likely various culicine mosquitoes (Diptera: Culicidae). In general, as more information becomes available on species morphology, biological variability, distribution, and natural host range of this group of filariids, the diagnostic significance of certain characters used to separate genera and species will become better understood. A revised simplified key to the genera is presented in light of this new addition to the subfamily.

Key to the Genera of the Subfamily Waltonellinae

- 1a. Cuticularized parastomal structures present 2
- 1b. Cuticularized parastomal structures absent *Paramadochotera* (Madagascar)
- 2a. Lateral and caudal alae present 3
- 2b. Lateral and caudal alae absent 4
- 3a. Cuticle with transversely oriented ridges and bosses ... *Madochotera* (Madagascar)
- 3b. Cuticle smooth, generally lacking bosses *Foleyellides* (worldwide)

- 4a. Cuticle of midbody with annular bands of longitudinally oriented bosses; microfilaria sheathed with tip of tail rounded, often bulbous
..... *Ochoterenella* (Neotropics)
- 4b. Cuticle of midbody with scattered minute bosses; microfilaria unsheathed with tip of tail slightly narrowed
..... *Paraochoterenella* (Indonesia)

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